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Two new species of *Pluteus* section *Celluloderma* from the Dominican Republic

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Abstract— Two new species of *Pluteus* sect. *Celluloderma* collected in the Dominican Republic are described based on morphological and molecular (nrITS) characters. *Pluteus crenulatus* is characterized by the sulcate crenulate margin of the pileus, clavate cheilo- and caulocystidia, and absence of pleurocystidia. *Pluteus stenotrichus* is characterized by the presence of very narrow and internally septate cells in the pileipellis.

Key words— biodiversity, Caribbean, phylogeny, *Pluteaceae*, taxonomy

Introduction

The genus *Pluteus* Fr. (*Pluteaceae, Agaricales*) has been the focus of recent molecular phylogenetic studies (Menolli et al. 2010; Justo et al. 2011a,b), which essentially support the traditional subdivision of the genus in three sections (*Pluteus*, *Celluloderma* Fayod, and *Hispidoderma* Fayod) but with some rearrangements. Sect. *Celluloderma* still accommodates all species with non-metuloid hymenial cystidia and a pileipellis arranged as an hymeniderm or epithelium composed mostly of clavate or spheropedunculate elements (intermixed or not with elongated cells) but now, based on molecular data, also includes taxa with a cutis-type pileipellis (previously assigned to sect. *Villosi* Schreurs & Vellinga or *Hispidoderma*) or with distinct partial veil (previously assigned to *Chamaeota* (W.G. Sm.) Earle) (Menolli et al. 2010; Justo et al. 2011a,b; Vizzini & Ercole 2011).

Here we describe two new *Pluteus* species recently collected in the Dominican Republic. Morphological and molecular (nrITS) data support their inclusion in sect. *Celluloderma*. Both taxa are compared with morphologically similar species and their phylogenetic position is discussed.

Materials & methods

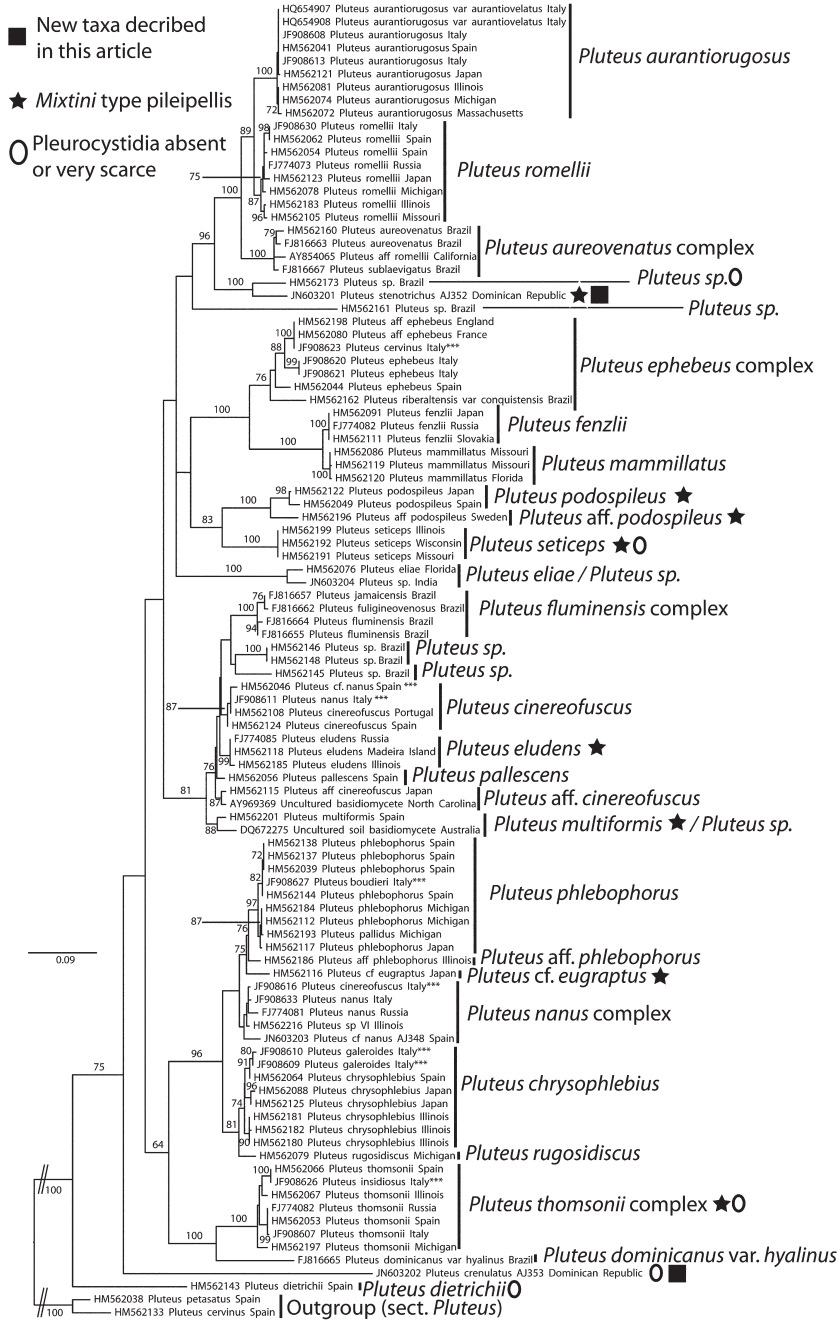
Collections were studied using standard procedures for morphological examination of *Pluteus* (e.g., Justo & Castro 2007, Minnis & Sundberg 2010). Descriptive morphological terms follow Vellinga (1988). The notation [30, 2, 1] indicates that measurements were made of 30 basidiospores from 2 basidiocarps in 1 collection. Color codes are from Munsell Soil-Color Charts (Munsell Color 2009). The following abbreviations are used in the descriptions: avl for average length, avw for average width, Q for the length/width quotient and avQ for average quotient.

Standard procedures for DNA isolation, PCR, and sequencing were applied (e.g., Justo et al. 2011a,b). The "nuclear ribosomal internal transcribed spacers 1 and 2 including the 5.8S region" is abbreviated as nrITS. The final dataset includes sect. *Celluloderma* nrITS sequences generated by Justo et al. (2011b), additional sequences generated by the first author, and sequences currently available in GenBank (mostly originated by O'Brien et al. 2005, Matheny et al. 2006, Midgley et al. 2007, Malysheva et al. 2009, Menolli et al. 2010). All accession numbers and sequence geographic origins are given in Fig. 1. Sequences were aligned using MAFFT version 6 (http://mafft.cbrc.jp/alignment/server/; Katoh & Toh 2008) with the Q-INS-i option, and alignments were manually corrected using MacClade 4.05 (Maddison & Maddison 2002) before deposition in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S12007). A Maximum Likelihood analysis was run using the RAxML servers with 100 rapid bootstrap replicates (http://phylobench.vital-it.ch/raxml-bb/index.php; Stamatakis et al. 2008). *Pluteus cervinus* (Schaeff.) P. Kumm. and *P. petasatus* (Fr.) Gillet (both sect. *Pluteus*) were used as outgroup taxa.

Results

The phylogenetic position of the new taxa described here is highlighted in Fig. 1 and discussed in detail under Taxonomy. Sequence identifications marked with "***" (which require further attention but fall outside the scope of this paper) are listed here to avoid causing further taxonomic confusion in GenBank: (i) "*Pluteus* cf. *nanus*" (HM562046) and "*P. nanus*" (JF908611) probably represent *P. cinereofuscus* J.E. Lange; (ii) "*Pluteus cinereofuscus*" (JF908616) is part of the *Pluteus nanus* (Pers.) P. Kumm. species complex; (iii) "*Pluteus cervinus*" (JF908623) represents a taxon close to *Pluteus ephebeus* (Fr.) Gillet; (iv) "*Pluteus galeroides*"(JF908610, JF908609) probably represents *Pluteus chrysophlebius* (Berk. & M.A. Curtis) Sacc.; (v) "*Pluteus boudieri*" (JF908627) represents *Pluteus phlebophorus* (Ditmar) P. Kumm.; (vi) *Pluteus insidiosus* (JF908626) falls within collections morphologically assignable to *Pluteus thomsonii* (Berk. & Broome) Dennis.

Figure 1. Best tree from the Maximum Likelihood analysis of *Pluteus* sect. *Celluloderma*. Bootstrap values ≥70% are shown on or below the branches. Root length has been reduced to facilitate graphical representation.


New taxa decribed in this article
Mixtini type pileipellis
Pleurocystidia absent or very scarce
HQ654907 Pluteus aurantiorugosus var aurantiovelatus Italy
HQ654908 Pluteus aurantiorugosus var aurantiovelatus Italy
JF908608 Pluteus aurantiorugosus Italy
HM562041 Pluteus aurantiorugosus Spain
JF908613 Pluteus aurantiorugosus Italy
HM562121 Pluteus aurantiorugosus Japan
HM562081 Pluteus aurantiorugosus Illinois
HM562074 Pluteus aurantiorugosus Michigan
HM562072 Pluteus aurantiorugosus Massachusetts
Pluteus aurantiorugosus
JF908630 Pluteus romellii Italy
HM562062 Pluteus romellii Spain
HM562054 Pluteus romellii Spain
FJ774073 Pluteus romellii Russia
HM562123 Pluteus romellii Japan
HM562078 Pluteus romellii Michigan
HM562183 Pluteus romellii Illinois
HM562105 Pluteus romellii Missouri
Pluteus romellii
HM562160 Pluteus aureovenatus Brazil
FJ816663 Pluteus aureovenatus Brazil
AY854065 Pluteus aff romellii California
FJ816667 Pluteus sublaevigatus Brazil
Pluteus aureovenatus complex
HM562173 Pluteus sp. Brazil
Pluteus sp.
JN603201 Pluteus stenotrichus AJ352 Dominican Republic
HM562161 Pluteus sp. Brazil
Pluteus sp.
HM562198 Pluteus aff ephebeus England
HM562080 Pluteus aff ephebeus France
JF908623 Pluteus cervinus Italy***
JF908620 Pluteus ephebeus Italy
JF908621 Pluteus ephebeus Italy
HM562044 Pluteus ephebeus Spain
HM562162 Pluteus riberaltensis var conquistensis Brazil
Pluteus ephebeus complex
HM562091 Pluteus fenzlii Japan
FJ774082 Pluteus fenzlii Russia
HM562111 Pluteus fenzlii Slovakia
Pluteus fenzlii
HM562086 Pluteus mammillatus Missouri
HM562119 Pluteus mammillatus Missouri
HM562120 Pluteus mammillatus Florida
Pluteus mammillatus
HM562122 Pluteus podospileus Japan
HM562049 Pluteus podospileus Spain
Pluteus podospileus
HM562196 Pluteus aff podospileus Sweden
Pluteus aff. podospileus
HM562199 Pluteus seticeps Illinois
HM562192 Pluteus seticeps Wisconsin
HM562191 Pluteus seticeps Missouri
Pluteus seticeps
HM562076 Pluteus eliae Florida
JN603204 Pluteus sp. India
Pluteus eliae / Pluteus sp.
FJ816657 Pluteus jamaicensis Brazil
FJ816662 Pluteus fuligineovenosus Brazil
FJ816664 Pluteus fluminensis Brazil
FJ816655 Pluteus fluminensis Brazil
Pluteus fluminensis complex
HM562146 Pluteus sp. Brazil
HM562148 Pluteus sp. Brazil
Pluteus sp.
HM562145 Pluteus sp. Brazil
Pluteus sp.
HM562046 Pluteus cf. nanus Spain ***
JF908611 Pluteus nanus Italy ***
HM562108 Pluteus cinereofuscus Portugal
HM562124 Pluteus cinereofuscus Spain
Pluteus cinereofuscus
FJ774085 Pluteus eludens Russia
HM562118 Pluteus eludens Madeira Island
HM562185 Pluteus eludens Illinois
Pluteus eludens
HM562056 Pluteus pallescens Spain
Pluteus pallescens
HM562115 Pluteus aff cinereofuscus Japan
AY969369 Uncultured basidiomycete North Carolina
Pluteus aff. cinereofuscus
HM562201 Pluteus multiformis Spain
DQ672275 Uncultured soil basidiomycete Australia
Pluteus multiformis / Pluteus sp.
HM562138 Pluteus phlebophorus Spain
HM562137 Pluteus phlebophorus Spain
HM562039 Pluteus phlebophorus Spain
JF908627 Pluteus boudieri Italy***
HM562144 Pluteus phlebophorus Spain
HM562184 Pluteus phlebophorus Michigan
HM562112 Pluteus phlebophorus Michigan
HM562193 Pluteus pallidus Michigan
HM562117 Pluteus phlebophorus Japan
Pluteus phlebophorus
HM562186 Pluteus aff phlebophorus Illinois
Pluteus aff. phlebophorus
HM562116 Pluteus cf eugraptus Japan
Pluteus cf. eugraptus
JF908616 Pluteus cinereofuscus Italy***
JF908633 Pluteus nanus Italy
FJ774081 Pluteus nanus Russia
HM562216 Pluteus sp VI Illinois
JN603203 Pluteus cf nanus AJ348 Spain
Pluteus nanus complex
JF908610 Pluteus galeroides Italy***
JF908609 Pluteus galeroides Italy***
HM562064 Pluteus chrysophlebius Spain
HM562088 Pluteus chrysophlebius Japan
HM562125 Pluteus chrysophlebius Japan
HM562181 Pluteus chrysophlebius Illinois
HM562182 Pluteus chrysophlebius Illinois
HM562180 Pluteus chrysophlebius Illinois
Pluteus chrysophlebius
HM562079 Pluteus rugosidiscus Michigan
Pluteus rugosidiscus
HM562066 Pluteus thomsonii Spain
JF908626 Pluteus insidiosus Italy***
HM562067 Pluteus thomsonii Illinois
FJ774082 Pluteus thomsonii Russia
HM562053 Pluteus thomsonii Spain
JF908607 Pluteus thomsonii Italy
HM562197 Pluteus thomsonii Michigan
Pluteus thomsonii complex
FJ816665 Pluteus dominicanus var hyalinus Brazil
Pluteus dominicanus var. hyalinus
JN603202 Pluteus crenulatus AJ353 Dominican Republic
HM562143 Pluteus dietrichii Spain
Pluteus dietrichii
HM562038 Pluteus petasatus Spain
HM562133 Pluteus cervinus Spain
Outgroup (sect. Pluteus)
0.09

Taxonomy

Pluteus crenulatus Justo, Battistin & Angelini, **sp. nov.** FIG. 2

MYCOBANK 563469

Similar to *Pluteus tucumanus*, also with a sulcate pileus with crenulate margin, but differing in broader spores, clavate cheilo- and caulocystidia, and absence of pleurocystidia

TYPE: Dominican Republic, Prov. Puerto Plata, Sosua, Unión, 17 Jan 2011, leg. C. Angelini, coll. AJ353 (**Holotype** MICH; GenBank nrITS JN603202. **Isotype** MCVE 27198).

ETYMOLOGY: *crenulatus* refers to the distinctive crenulate pileus margin.

PILEUS 20–40 mm, convex to plano-convex, with a broad umbo, deeply sulcate in the outer half; surface smooth or minutely granulose at center, granulose towards the margin; brown, darker at center [7.5YR 3/4, 4/6] and paler towards margin [7.5YR 5/6, 5/8, 6/6] where it alternates brown and whitish zones, not hygrophanous, margin distinctly crenulate, white. LAMELLAE crowded, free, ventricose; ≤ 4 mm broad; white when young, later pink with flocculose whitish edges. STIPE 30–50 × 2–4 mm, cylindrical, slightly broadened at base; white or white-cream; smooth or pruinose. CONTEXT in stipe and pileus white. SMELL indistinct. TASTE not recorded. SPORE PRINT not recorded, probably pink.

BASIDIOSPORES [30, 2, 1] 4.5–5.5(–6.0) × 3.9–5.0(–5.5) µm, avl × avw = 4.9 × 4.2 µm, Q = 1.00–1.26, avQ = 1.17, globose to broadly ellipsoid. BASIDIA 20–35 × 9–12 µm, 4-spored, clavate or narrowly utriform. PLEUROCYSTIDIA absent. LAMELLAR EDGE sterile, completely covered with cystidia. CHEILOCYSTIDIA 24–73 × 12–34 µm, (narrowly) clavate, a few narrowly utriform; colorless; with thin, smooth walls; crowded, forming a well-developed strip. PILEIPELLIS a hymeniderm with transitions towards and epithelium; individual elements 29–61 × 14–49 µm, mostly clavate or spheropedunculate, a few narrowly utriform, mucronate, or with basal septum; filled with brown intracellular pigment, evenly dissolved or more rarely with pigment condensations; with thin, smooth walls. STIPITIPELLIS a cutis; hyphae 4–13 µm wide, cylindrical, colorless or with brown pigment; with thin, smooth walls. CAULOCYSTIDIA 38–51 × 17–31 µm, (narrowly) clavate, some multiseptate; hyaline or with brown intracellular pigment, some with additional incrusting pigment; with thin to slightly thickened (less than 1 µm), smooth walls; all over stipe surface in tightly packed clusters. CLAMP CONNECTIONS absent in all tissues.

HABITAT AND DISTRIBUTION—Gregarious, on a fallen log in broad-leaved forest, January. Known only from the type locality in the Dominican Republic.

COMMENTS—The deeply sulcate pileus with a crenulate margin is the most distinctive macroscopic feature of *P. crenulatus*. Microscopically, it is characterized by the clavate cheilo- and caulocystidia (FIG. 2) and the absence of pleurocystidia.

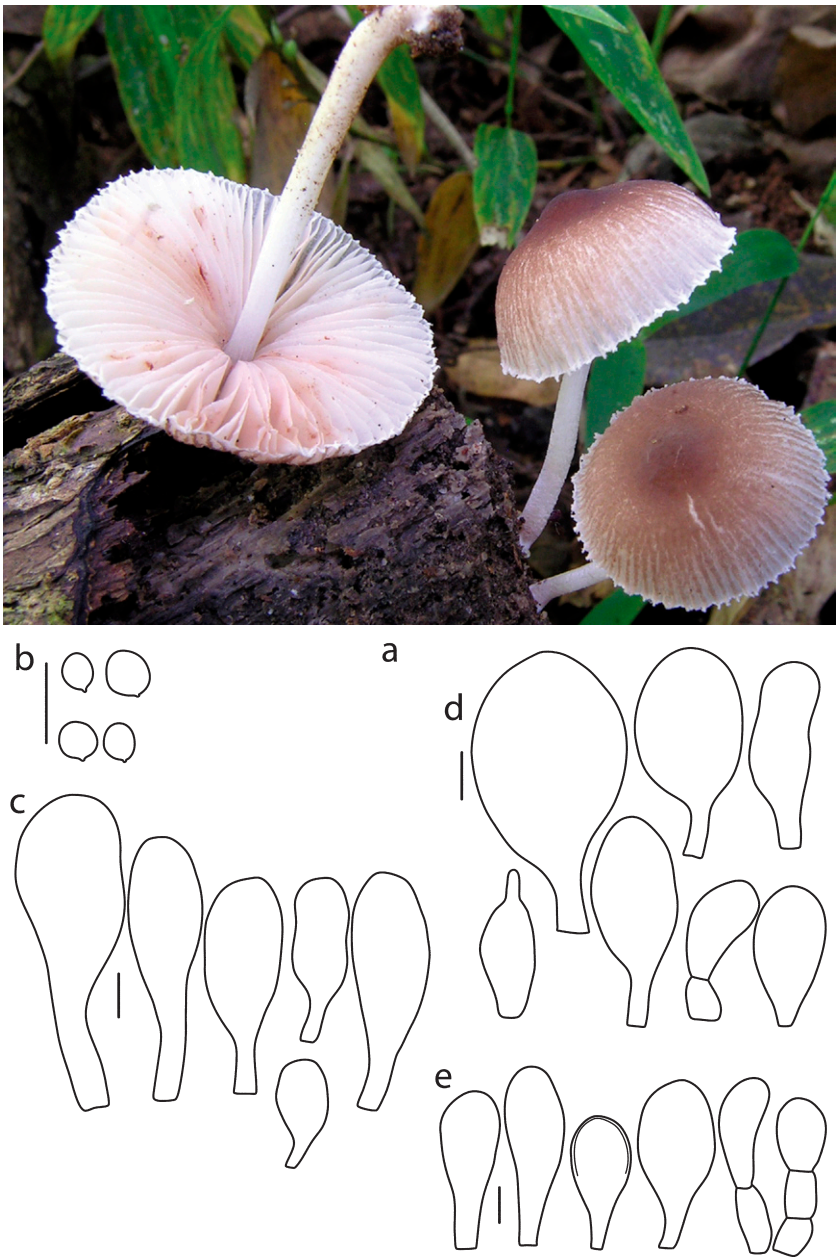


Figure 2. *Pluteus crenulatus* (holotype AJ353). a. Basidiocarps; b. Basidiospores; c. Cheilocystidia; d. Pileipellis elements; e. Caulocystidia. Scale bars = 10 μm.

P. tucumanus is a South American species that also has a sulcate, appendiculate-crenate pileus (Singer & Digilio 1952; Singer 1956, 1958). The following observations are based on our examination of its isotype in MICH (Singer T921 Argentina, Prov. Tucumán, Rio de los Sosas, 18 Jan 1950):

Pluteus tucumanus Singer, Lilloa 25: 269. 1952 ["1951"]. Fig. 3

Basidiospores [30, 1, 1] 3.9–5.0 × 2.7–4.0 µm, avl × avw = 4.5 × 3.6 µm, Q = 1.00–1.34(–1.69), avQ = 1.26, globose to ellipsoid, very rarely oblong. Basidia 25–31 × 7–10 µm, 4-spored, narrowly utriform. Pleurocystidia 40–53 × 18–22 µm, (narrowly) utriform with a distinct pedicel; colorless; with thin, smooth walls; scarce and scattered, present all over lamellar sides. Lamellar edge sterile, completely covered with cystidia. Cheilocystidia 29–52 × 12–32 µm, (narrowly) utriform or broadly fusiform, a few narrowly clavate; colorless; with thin, smooth walls; crowded, forming a well-developed strip. Pileipellis an hymeniderm with transitions towards and epithelium; individual elements 38–49 × 21–32 µm, mostly clavate or spheropedunculate; filled with brown intracellular pigment, evenly dissolved or more rarely with pigment condensations; with thin, smooth walls. Stipitipellis a cutis; hyphae 5–13 µm wide, cylindrical, colorless or with brown pigment; with thin, smooth walls. Caulocystidia 38–51 × 17–31 µm, (narrowly) clavate to (narrowly) utriform; hyaline or with brown intracellular pigment; with thin, smooth walls; all over stipe surface, scattered or in clusters. Clamp–connections absent in all tissues.

Although the differences between *P. crenulatus* and *P. tucumanus* are subtle (the latter distinguished by narrower spores, presence of pleurocystidia, and differently shaped cheilo- and caulocystidia), such subtle variations have proved critical in separating species in sect. *Celluloderma* (Justo et al. 2011c).

Singer (1958) placed *Pluteus fallax* Singer and *P. iguazuensis* Singer into the same morphological group (stirps *Tucumanus*) as *P. tucumanus*. We were unable to examine either species, but based on Singer's (1958) descriptions both taxa differ from *P. crenulatus* in (among other characters) the non-crenulate pileus margin and presence of pleurocystidia. Singer also emphasized the presence of pigment condensations in the pileipellis elements of *P. tucumanus* and the absence of such condensations in *P. fallax* and *P. iguazuensis*. We observed pigment condensations in both *P. crenulatus* and *P. tucumanus*, but the taxonomic significance of this feature needs further study.

Macroscopically, *P. crenulatus* does not resemble any species in sect. *Celluloderma* with very rare to absent pleurocystidia — e.g., *P. diettrichii* Bres., *P. insidiosus* Vellinga & Schreurs, *P. poliocnemis* Kühner — which also differ microscopically in spore and cystidia shape and size (see Kühner & Romagnesi (1956) and Vellinga (1990) for full descriptions). *Pluteus thomsonii*

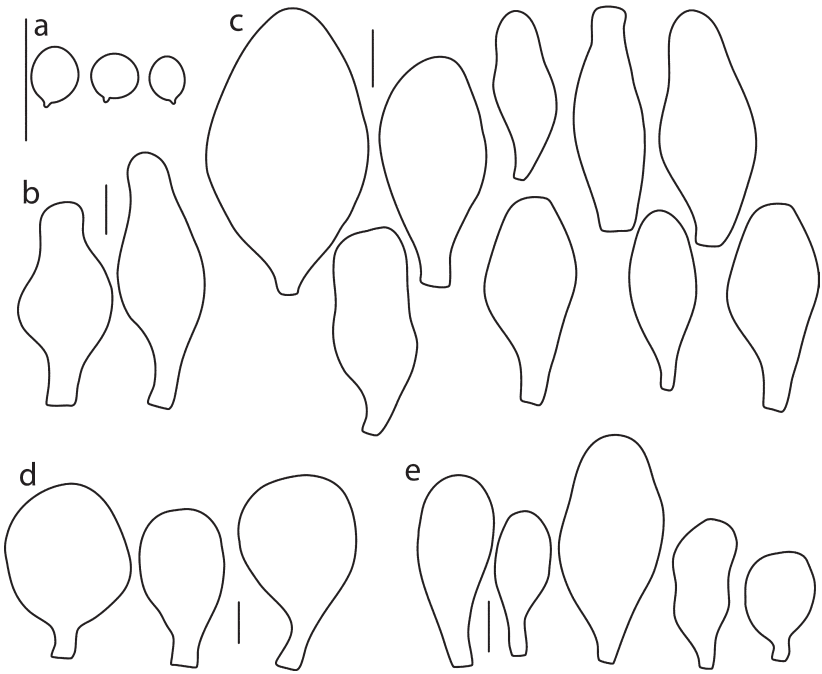


Figure 3. *Pluteus tucumanus* (isotype, Singer T921). a. Basidiospores; b. Pleurocystidia; c. Cheilocystidia; d. Pileipellis elements; e. Caulocystidia. Scale bars = 10 µm.

and *P. seticeps* (G.F. Atk.) Singer, which sometimes have very rare or absent pleurocystidia, differ from *P. crenulatus* by the "*Mixtini*-type" pileipellis (see Minnis & Sundberg (2010) and Vellinga (1990) for full descriptions).

Phylogenetically, *P. crenulatus* appears somewhat isolated as sister to all taxa in sect. *Celluloderma* except *P. diettrichii* Bres. (Fig. 1). This placement receives no statistical support but suggests that *P. crenulatus* is not closely related to any of the major lineages presently recognized in sect. *Celluloderma* (Justo et al. 2011b).

Pluteus stenotrichus Justo, Battistin & Angelini, **sp. nov.** Fig. 4

MycoBank 563470

Characterized by a "*Mixtini*-type" pileipellis with very narrow, internally septate cylindrical elements.

Type: Dominican Republic, Prov. Puerto Plata, Sosua, El Castillo, 16 Jan 2011, leg. C. Angelini, coll. AJ352 (**Holotype** MICH; GenBank nrITS JN603201. **Isotype** MCVE 27197).

Etymology: *stenotrichus* (= "with narrow hairs") refers to the very unusual type of pileipellis cells.

Pileus (20–)25–50(–60) mm, convex to plano-convex, with or without a low broad umbo; surface distinctly rugose-venose at and around center, radially areolate-rimose in the outer half, exposing the white context; brown, darker at center [7.5YR 2.5/2, 2.5/3, 3/2, 3/3], paler around center [7.5YR 4/6, 5/6, 5/8] and much paler at margin [7.5YR 7/4, 7/6, 7/8], not hygrophanous; margin rimose but not striate or sulcate. Lamellae crowded, free, ventricose; ≤ 7 mm broad; white or cream when young, later pink [5YR 8/3, 8/4], with concolorous or paler edges. Stipe 30–60 × 4–6 mm, cylindrical, slightly broadened at base; white or white-cream; smooth or slightly pruinose. Context in stipe and pileus white. Smell indistinct. Taste not recorded. Spore print not recorded, probably pink.

Basidiospores [30, 2, 1] 4.9–7.0 × 4.5–5.7 µm, avl × avw = 5.6 × 5.1 µm, Q = 1.00–1.18, avQ = 1.11, globose to broadly ellipsoid. Basidia 25–34 × 7.5–10 µm, 4-spored, clavate or narrowly utriform. Pleurocystidia 34–83 × 14–32 µm, (narrowly) utriform or lageniform, a few broadly conical with long pedicel; colorless; with thin, smooth walls; relatively common, scattered over lamellar sides. Lamellar edge sterile, completely covered with cystidia. Cheilocystidia 25–50(–65) × 14–20(–25) µm, (narrowly) clavate, spheropedunculate, some broadly fusiform, some with basal septum; colorless; with thin, smooth walls; crowded, forming a well-developed strip. Pileipellis a hymeniderm with transitions towards and epithelium, composed of three types of elements: (i) globose, clavate or spheropedunculate 31–76 × 22–60 µm; (ii) narrowly clavate or lageniform 53–88 × 15–29 µm; (iii) vey narrow elongated elements, 60–123 × 7–10(–12) µm, cylindrical or flexuous, with 0–4 internal septa, usually arising from a basal element ≤ 15 µm wide; all elements filled with brown intracellular pigment, more rarely hyaline; with thin, smooth walls. Stipitipellis a cutis; hyphae 5–15 µm wide, cylindrical, colorless or with brown pigment; with thin, smooth walls. Caulocystidia 31–63 × 12–24 µm, (narrowly) clavate, narrowly utriform or conical with basal septum; hyaline or with brown intracellular pigment; with thin, smooth walls; scattered all over stipe surface, isolated or in loosely arranged clusters. Clamp connections absent in all tissues.

Habitat and distribution—Gregarious, apparently terrestrial (among woody remnants and organic matter) in broad-leaved forest, January. Known only from the type locality in the Dominican Republic.

Comments—The very narrow and internally septate elements in the pileipellis (Fig. 4) are diagnostic for *P. stenotrichus*. Taxa with both clavate-spheropedunculate and elongated elements in the pileipellis have been traditionally classified in subsect. *Mixtini* Singer, but molecular data suggest that elongated elements in the pileipellis have evolved several times within sect. *Celluloderma*, rendering subsect. *Mixtini* artificial (Justo et al. 2011a,

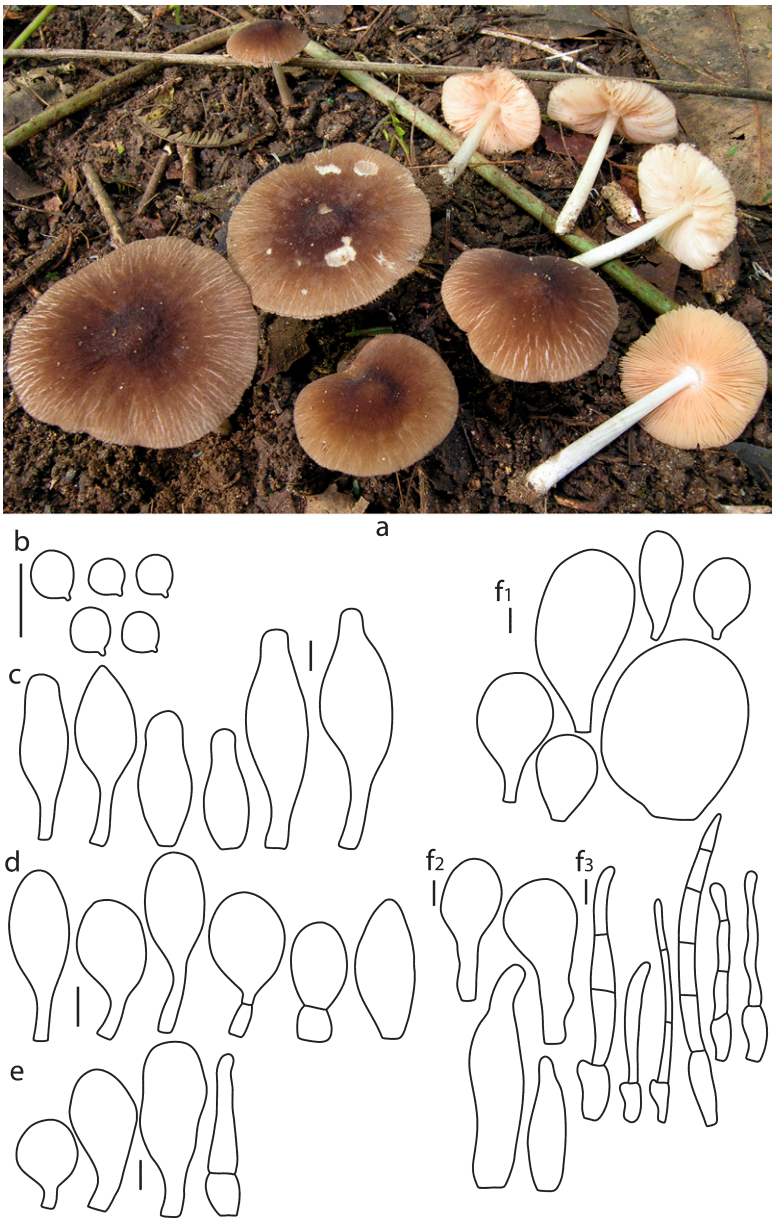


Figure 4. *Pluteus stenotrichus* (holotype AJ352). a. Basidiocarps; b. Basidiospores; c. Pleurocystidia; d. Cheilocystidia; e. Caulocystidia; f. Pileipellis elements – f1 clavate and spheropedunculate, f2 narrowly clavate and lageniform, f3 very narrow and internally septate. Scale bars = 10 µm.

Fig. 1). An extensive literature search and comparison with all species described with a "*Mixtini*-type" pileipellis (e.g. Horak 1964, 2008; Horak & Heinemann 1978; Minnis & Sundberg 2010; Orton 1986; Pegler 1977, 1986; Singer 1956, 1958; Vellinga 1990; see also Justo et al. 2011a,b,c for additional taxonomic references) found none with this type of septate elongated element.

Pluteus stenotrichus is not closely related to any previously sequenced species with a "*Mixtini*-type" pileipellis (Fig. 1) and its position in the *romellii/aurantiorugosus* group is rather unexpected based on morphology. *Pluteus aurantiorugosus* (Trog) Sacc., *P. romellii* (Britzelm.) Sacc., and taxa in the species complex around *P. aureovenatus* Menolli & Capelari all lack any elongated pileipellis elements and are characterized in part by the bright yellow or orange-red colors of the pileus and/or stipe. The closest relative of *P. stenotrichus* is an undescribed species from Brazil (nrITS HM562173, collection SP394383) but that taxon differs in the (among other characters) absence of elongated pileipellis elements and almost total absence of hymenial cystidia (N. Menolli Jr. & M. Capelari, pers. comm.).

The narrow septate elements in the pileipellis of *P. crenulatus* are reminiscent of those typically exhibited by some members of sect. *Hispidoderma* (e.g., *P. exiguus* (Pat.) Sacc.). However the general organization of the pileipellis differs in those taxa and lacks the well-developed hymeniform layer made up of clavate or spheropedunculate elements that is typical of *P. stenotrichus* and the other species with "*Mixtini*-type" pileipellis in sect. *Celluloderma*.

Acknowledgments

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